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Thawing times and haemostatic assessment of fresh frozen plasma thawed at 37°C and 45°C using water-bath methods

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Abstract

BACKGROUND: The Barkey Plasmatherm (BP) can thaw plasma at 37°C and 45°C. No studies have assessed thawing times or haemostatic qualities of plasma thawed at 45°C with BP. This study assessed FFP thawing times using BP at 37°C and 45°C and Thermogenesis Thermoline (TT) at 37°C, and compared haemostatic quality of LG-Octaplas using BP at 37°C and 45°C with TT at 37°C.

STUDY DESIGN AND METHODS: Thawing time of FFP (pairs or fours) was assessed using BP at 37°C and 45°C (not pre-warmed, and pre-warmed) and TT at 37°C. Haemostasis was assessed in LG-Octaplas five minutes, 24 hours, 48 hours, and 120 hours post thaw using the three methods.

RESULTS: Thawing time for two units was 13.44' using TT, the same as using BP at 37°C (12.94' not pre-warmed; 12.20' pre-warmed) or 45°C (12.38' not pre-warmed), but longer than using BP pre-warmed to 45°C (11.31', $p<0.001$). Thawing time for four units was 13.41' using TT, shorter than using BP at 37°C (17.19' not pre-warmed; 18.47' pre-warmed; both $p<0.001$) or 45°C (15.03' not pre-warmed, $p=0.012$; 15.22' pre-warmed, $p=0.004$). There was no reduction in haemostatic markers in LG-Octaplas using BP at 37°C or 45°C compared to TT.

CONCLUSION: BP is quicker than TT by two minutes when thawing two units of FFP if it is pre-warmed to 45°C. BP is slower than TT by at least two minutes when thawing four units of FFP. There was no significant difference in the haemostatic qualities of plasma whether thawed at 37°C or 45°C.

Keywords: Plasma thawing; haemostasis; water-bath

Introduction

In the last decade there has been an increase interest in transfusing fresh frozen plasma (FFP) and cryoprecipitate early for the treatment of massive haemorrhage, with clinical trials suggesting that this practice improves outcome for patients who are bleeding due to trauma.[1,2] In order to improve the availability of plasma components for management of bleeding, many countries have now extended the shelf life of thawed FFP from 24 hours to 5 days for thawed plasma and 14 days or more for liquid plasma.[3-5] Although there have been no studies demostrating a clinical difference between freshly thawed plasma, extended shelf life thawed plasma or liquid plasma, and the PROPPR study[1] used extended shelf life thawed plasma, *in vitro* data on thawed and liquid plasma has demonstrated that the haemostatic qualities of the components deteriorate over time.[6] An optimal situation may be to have faster thawing methods that would allow for quick delivery of thawed plasma on demand, providing fresh components for patients. Fast thawing may also be desirable when stocks of thawed plasma are rapidly depleted, especially when multiple patients are being treated concurently, or in centres where plasma is not often required and pre-thawing may lead to increased wastage.

There are several methods available for thawing FFP including water-bath, microwave oven, and dry-heat based methods: current national guidance in the United Kingdom for thawing plasma recommends that the optimal temperature at which the components should be thawed is 37°C (33°C – 37°C).[4,7]

Barkey Plasmatherm (Barkey GmbH & Co. KG, Leopoldshöhe, Germany) is a water-based method that can thaw plasma at 37°C and 45°C temperatures. However, to our knowledge there have been no studies that have assessed the thawing time or the haemostatic qualities of plasma when thawed at 45°C temperatures in comparison with other thawing methods. The aims of this study are to: a) assess the thawing times between Barkey Plasmatherm at 37°C and 45°C and Thermogenesis Thermoline (Helmer Scientific, Indiana, USA) at 37°C, using variable volumes of single donor FFP; and b) compare the effect of thawing temperatures on the haemostatic qualities of LG-Octaplas (a pooled plasma component) using Barkey Plasmatherm (BP) at 37°C and 45°C and Thermogenesis

Thermoline (TT) at 37°C. A pooled component rather than multiple units of FFP were used for the haemostatic assessment to reduce potential variation in clotting factors between units.

Materials and Methods

Fresh frozen plasma (FFP) was supplied by NHS Blood and Transplant (NHSBT) and was derived from UK whole blood donation (475mL ± 10%,) collected into 66.5mL CPD anticoagulant in top-and-top collection packs (FQE6283LB MacoPharma, Twickenham, UK) following NHSBT standard operation procedures.[8] LG-Octaplas was supplied by Octapharma as part of routine delivery to hospital and was produced according to the manufacturer's specification

Assessment of thawing times

For the assessment of thawing times, single expired FFP units in pairs or in fours were assessed eight times using: a) Barkey Plasmatherm (BP) at 37°C, b) BP at 45°C, and c) Thermogenesis Thermoline (TT) at 37°C. The above experiment was repeated twice: 1) with the BP machine being switched on for <30 minutes, and 2) with the BP machine being switched on for at least four hours prior to the experiment. Assessment of thawing times was performed by two biomedical scientists using visual inspection for the absence of visible ice crystals, as this is the thawing end-point in most hospitals.

Assessment of haemostatic capacity

LG-Octaplas is a pooled plasma product that is likely to show less variability in haemostatic qualities than FFP, so 24 LG-Octaplas units (eight thawed in the TT at 37°C; eight thawed in the BP at 37°C; eight thawed in the BP at 45°C) were used for the assessment of haemostatic capacity stage of the experiment. A thawing time of 30 minutes at 37°C (30°C – 37°C) for LG-Octaplas is recommended by Octapharma Limited, and this timing was followed during the haemostatic assessment experiment for all three arms. After thawing, LG-Octaplas was stored at 2°C – 4°C and aliquots were taken for haemostatic testing at four different time-points: five minutes after removal from the thawing device (defined as time zero); 24 hours post thaw; 48 hours post thaw; and 120 hours post thaw – the latter timing

is the allowed extended shelf-life for thawed plasma in the UK.[4] Samples were stored below -70°C until testing was performed.[7]

Assays performed were prothrombin time (PT); activated partial thromboplastin time (APTT); fibrinogen; factors II, V, VII, VIII and XI activity; free protein S antigen; protein S activity; protein C activity; C1-inhibitor activity; and thrombin generation (TG). All assays except for thrombin generation were performed on a Sysmex CS-2100 analyser. PT was measured using Siemens Dade Innovin, and factor II, V and VII activity were measured by one-stage assays using Innovin and Siemens factor deficient plasmas; APTT was measured using Siemens Dade Actin FS, and factor XI was measured by one-stage assay using Actin FS and Siemens factor deficient plasma; factor VIII assays were measured using Hyphen Biomed Biophen VIII chromogenic reagents; protein C activity was measured using Siemens Berichrom Protein C reagents; free protein S antigen was measured using Siemens INNOVANCE Free PS reagents; protein S activity was measured using Hyphen Biomed Hemoclot Protein S reagents; C1-inhibitor activity was measured using reagents from Siemens; fibrinogen was measured by the Clauss method using Siemens Thrombin. All these assays (except for PT and APTT) were calibrated using Siemens Standard Plasma and all reagents were supplied by Sysmex UK (Milton Keynes, UK).

Thrombin generation was performed using the Calibrated Automated Thrombogram Fluoroskan system (Thrombinoscope BV, Maastricht, The Netherlands) as described by Hemker et al[9] in conjunction with the manufacturer's PPP reagents, which gave reaction concentrations of 5pM tissue factor and 4µM phospholipid. The following parameters of the TG curve were measured: lag time; time to the peak (ttP); peak thrombin; and area under the curve, known also as the endogenous thrombin potential (ETP).

Statistical Analysis

Data have been summarized as medians with 95% confidence intervals. Coagulation factors were analysed using univariate statistical methods. Analysis of variance (ANOVA) was performed, comparing concentration of each factor across arms and over time. For thawing times, Fisher's multiple comparisons test was used; for haemostatic assays Tukey's multiple

comparisons test was used. For all tests a p value of below 0.05 indicated a statistically significant difference.

Results

There was no statistically significant difference in the volumes of FFP thawed in each arm of the experiment (Figure 1).

Thawing times

Data are summarised in Figure 1.

For every increase in volume of 1mL there was a mean increase in thawing times for two units of 3.1 seconds at 37°C (TT or BP) and 1.9 seconds at 45°C; for four units the mean increase was 2.5 seconds (TT at 37°C and BP at 45°C) and 2.8 seconds (BP at 37°C).

The thawing time for two units of FFP using the TT was 13.44 minutes (95% confidence interval (CI) 11.78-15.10). The thawing time for two units of FFP using the BP at 37°C with pre-warming was 12.20 minutes (95% CI 11.77-12.63) compared to 12.94 minutes (95% CI 11.09-14.79) without pre-warming. The thawing time for two units of FFP using the BP at 45°C without pre-warming was 12.38 minutes (95% CI 11.70-13.05). None of these times were statistically significantly different from each other.

The thawing time for two units of FFP using the BP at 45°C with pre-warming was 11.31 minutes (95% CI 10.94-11.69); this was statistically significantly shorter than the thawing time using the TT by 2.13 minutes (p=0.030).

The thawing time for four units of FFP using the TT was 13.41 minutes (95% CI 12.81-14.01) which was not statistically significantly different from the thawing time for two units of FFP using the TT. When using the BP to thaw four units of FFP, the thawing time was statistically significantly longer than thawing in the TT whether using 37°C without pre-warming (17.19

minutes, 95% CI 16.21-18.16, p<0.001) or with pre-warming (18.47 minutes, 95% CI 17.78-19.16, p<0.001) or when using 45°C without pre-warming (15.03 minutes, 95% CI 14.20-15.86, p=0.012) or with pre-warming (15.22 minutes, 95% CI 14.44-16.00, p=0.004).

There was no statistically significant difference in thawing times using the BP whether the device was pre-warmed or not.

Haemostatic assays

Data are summarised in Figures 2 and 3.

For PT there was no statistically significant difference at time 0 between TT and BP at 37°C or BP at 45°C, and no statistically significant differences were seen within each thawing method at 24 hours, 48 hours or 120 hours. For all thawing methods, APTT showed a statistically significant increase over time but there was no statistically significant difference at all time points (time 0, 24 hours, 48 hours or 120 hours) between TT and BP at 37°C or 45°C. Similarly, for fibrinogen there was no statistically significant difference at all time points between the three thawing methods.

Factor VIII showed a statistically significant decrease over time regardless of thawing method but there was no statistically significant difference between TT and BP at 45°C at any time points, whereas factor VIII measured from the units thawed using the BP at 37°C was statistically significantly higher at time zero (median 76.0iu/dL) than when using the TT (median 57.7iu/dL, p=0.009); there was no statistically significant difference in factor VIII between units thawed at TT and BP at 37°C for any other time points. Factor V and protein S activity showed similar statistically significant decrease over time as for factor VIII but no statistically significant differences in results between TT and BP at 37°C or 45°C for any time points.

Endogenous thrombin potential was statistically significantly reduced over time following thawing, in line with the observed reduction of FVIII and FV. There was no statistically

significant difference at time 0 between TT and BP at 37°C or 45°C, and no statistically significant differences at 24 hours or 120 hours. Results for TT at 48 hours were statistically significantly lower than all other results: samples were run in the same batch as all samples for TT at 120 hours and a reason for this apparent anomaly could not be identified. Peak thrombin also statistically significantly reduced over time but there was no statistically significant difference at all time points between TT and BP at 37°C or 45°C.

There were no statistically significant differences between temperatures or time points for factor II, factor VII, factor XI, protein C activity, free protein S antigen or C1-inhibitor.

Discussion

The water bath method is the most commonly used method in the UK for thawing plasma, with the TT and BP being the most widely used devices. In this study we compared the two devices, and showed that with regards to thawing times TT was statistically significantly faster by at least two minutes when thawing four units of FFP, while BP at 45°C was statistically significantly faster by at least two minutes than TT when thawing two units of FFP. The reason for this is likely to be due to the difference in the contact surfaces area between the plasma units and the water between the two methods. In the TT the surface area is larger allowing for quicker thawing times, whereas in BP plasma units are placed close to each other thus, reducing the surface area with warm water and slowing down the thawing times.

In addition to thawing times, hospital transfusion laboratory who are considering the purchase of plasma thawing devices should also consider other factors, such as size, and maintenance of the device. For the two methods that we compared in this study, the TT is bigger in size than BP, allowing for up to ten units of plasmas being thawed at once – this can be a major advantage when dealing with mass casualties, however it will require more laboratory space to house. The BP can thaw a maximum of eight units (something that was not tested in this study), but four of these have to be placed below the cushions and need to be moved once first four units have been thawed and removed. Further, the cleaning and maintenance of TT is more demanding than BP: the TT requires the water to be changed regularly by laboratory staff, whereas the BP only requires the water to be replaced completely on an annual basis during the routine preventative maintenance; and in the TT spillages can enter the water, facilitating the need for complete replacement of the tank water, whereas the BP does not allow the water to come into contact with the product, as it uses a sealed tank method. Water is pumped into cushions and circulated. Should either the plasma bag or cushion rupture this is contained and easily cleaned.

In the UK, it is believed that temperatures close to 37°C may be optimal, and that thawing plasma at higher temperatures might affect the viability of plasma proteins.[4,10] The current UK recommendations state that plasma should be thawed at 33°C – 37°C.[4] However, to our

knowledge, there have been no studies that have compared BP at 45°C with 37°C thawing using other methods. The clinical need to have plasma available quickly for the management of major bleeding may have led to hospitals using a higher-than-recommended thawing temperature for plasma. While the extension of the shelf-life of thawed plasma has improved the availability of FFP in emergency situations, anecdotally this is still associated with some degree of wastage, particularly in hospitals that do not use a lot of plasma on a daily basis, and speeding up the thawing process for FFP could achieve a balance between reduced wastage and improved availability. In this study we observed that heat labile clotting factor levels and endogenous thrombin potential were statistically significantly reduced over time after thawing with both BP and TT devices: these findings are consistent with other studies[4,6] but the clinical impact of these reductions is not known. In this study, we did not see any statistically significant differences in any of the haemostatic markers of LG-Octaplas thawed at 45°C using BP compared to thawing at 37°C with BP or TT. Our results should give reassurance to clinicians and blood transfusion laboratories who are considering introducing a 45°C thawing temperature with the BP device, in that we found no evidence that this higher temperature of thawing for 30 minutes impairs the quality of plasma, but it should be noted that a limitation of our study is that the temperature of plasma after 30 minutes of thawing was not measured. There did not appear to be an advantage to leaving the BP switched on, suggesting that it can be switched on at the same time as thawing FFP is initiated. Further studies are required to validate our results.

In conclusion, compared to the TT, when thawing two units of FFP, the BP at 37°C is not statistically significantly different, but at 45°C is statistically significantly quicker by more than two minutes. When thawing four units of FFP the BP is statistically significantly slower than the TT by two minutes. These results were not statistically different whether the BP was pre-warmed or not. There was no statistically significant difference in the haemostatic qualities of plasma between 37°C and 45°C thawing temperatures.

Conflict of interest

Barkey Plasmatherm funded the reagent and plasma costs for performing the haemostatic assays in the second stage of the study. The views expressed in this document are those of the authors and not those of the company. The company had no role in the study design, data collection/analysis or preparation of this article.

Roles

SP and LG wrote the first draft of the paper. OE and JM performed the thawing experiments. OE and SP performed the haemostasis assays. All authors were involved in study design and contributed to the writing of the manuscript.

Figure 1: Comparison of FFP volumes and thawing times for 2 and 4 units thawed together (n=8). TT: Thermogenesis Thermoline; BP: Barkey Plasmatherm; *: 0.01<p<0.05; **: 0.001<p≤0.01; ***: 0.0001<p≤0.001; ****: p<0.0001. Box plots show median, 5th and 95th percentile and minimum and maximum values.

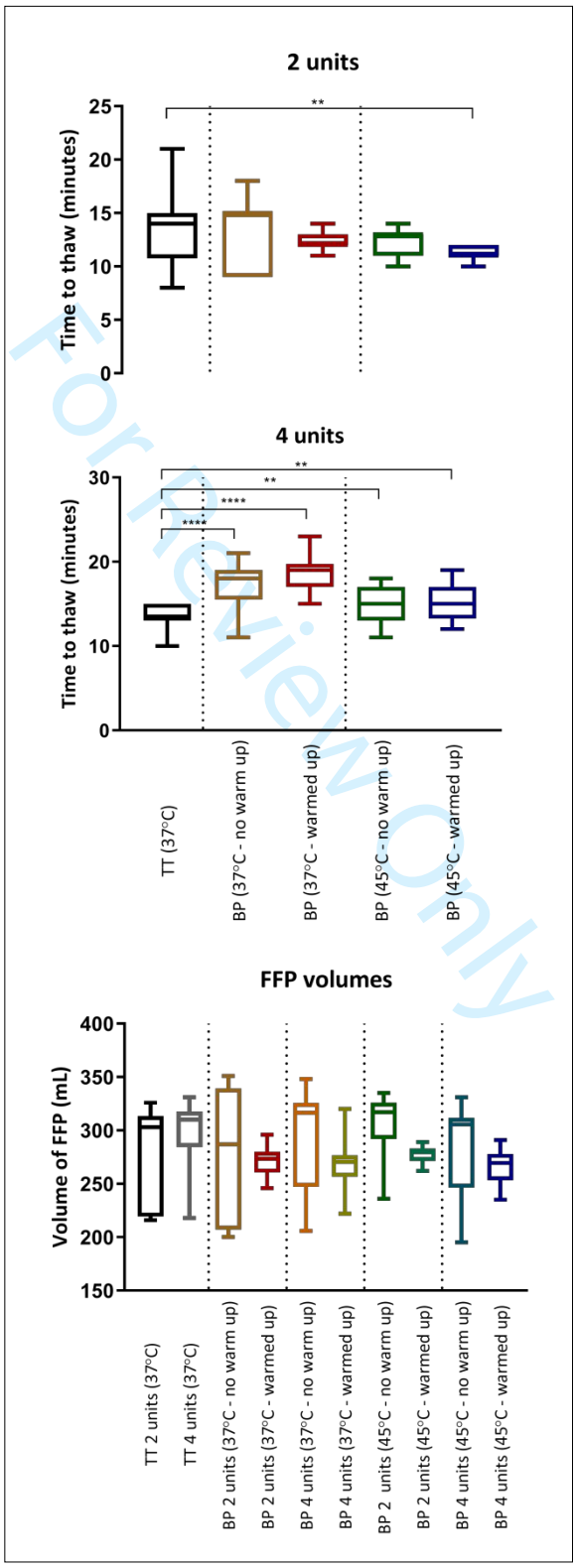

Figure 2: Comparison of clotting assays and thrombin generation in Octaplas following thawing by different methods. TT: Thermogenesis Thermoline; BP: Barkey Plasmtherm; PT: prothrombin time; APTT: activated partial thromboplastin time; ETP: endogenous thrombin potential. *: 0.01<p<0.05; **: 0.001<p≤0.01; ***: 0.0001<p≤0.001; ****: p<0.0001. Box plots show median, 5th and 95th percentile and minimum and maximum values.

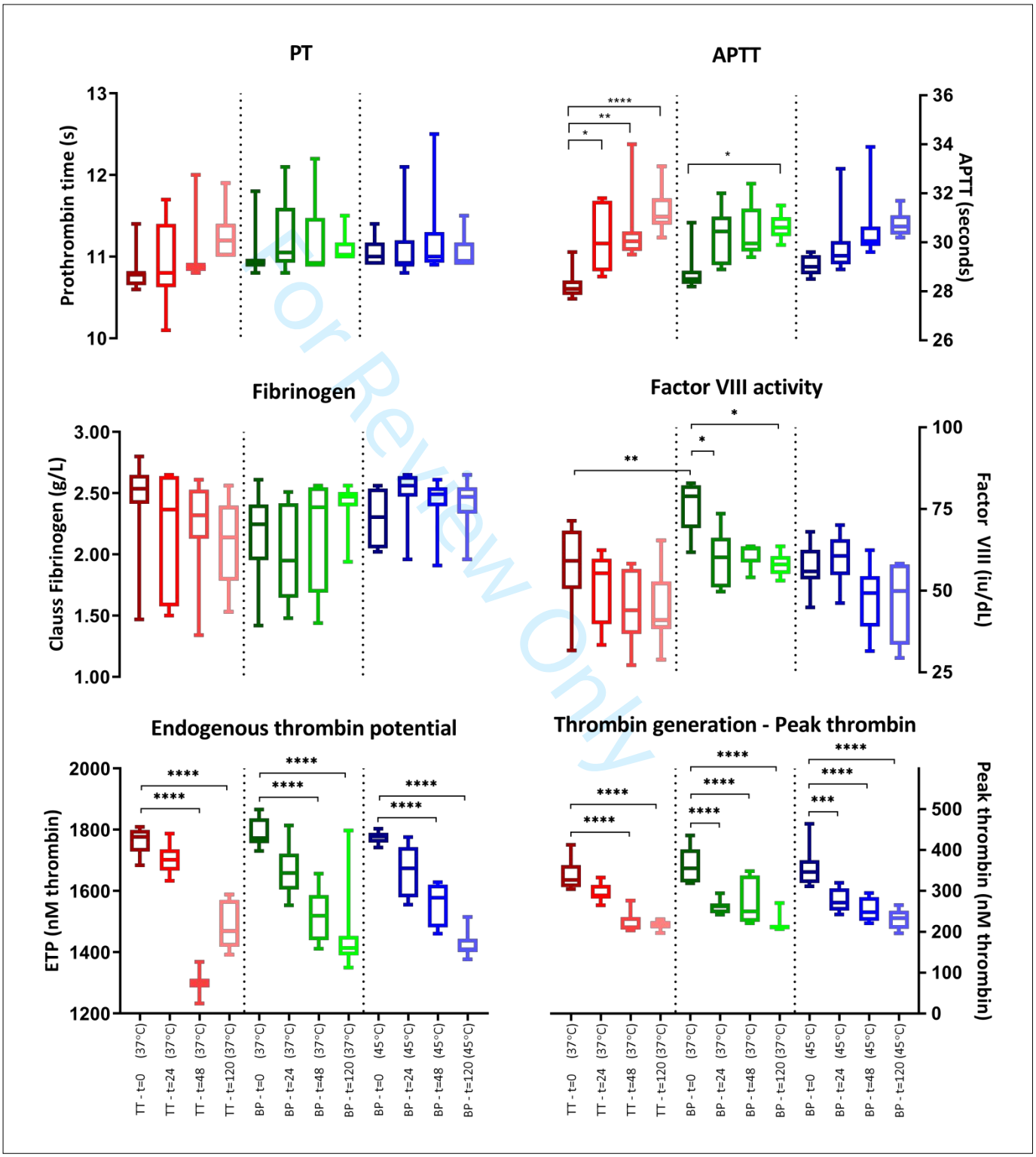

Figure 3: Comparison of haemostasis assays in LG-Octaplas following thawing by different methods. TT: Thermogenesis Thermoline; BP: Barkey Plasmatherm. Box plots show median, 5th and 95th percentile and minimum and maximum values.

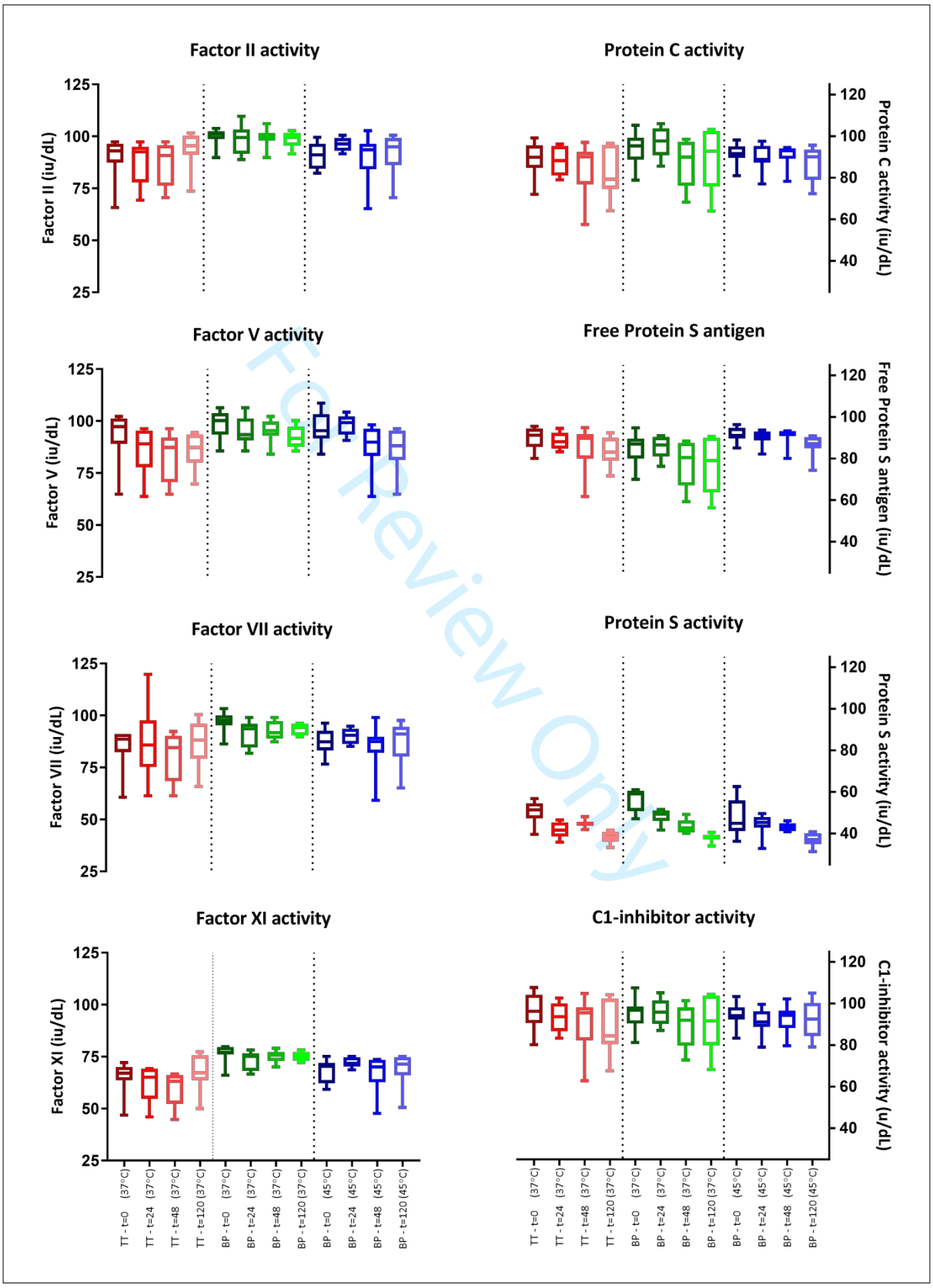

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Figure legends

Figure 1: Comparison of FFP volumes and thawing times for 2 and 4 units. TT: Thermogenesis Thermoline; BP: Barkey Plasmatherm; *: 0.01<p<0.05; **: 0.001<p≤0.01; ***: 0.0001<p≤0.001; ****: p<0.0001. Box plots show median, 5th and 95th percentile and minimum and maximum values.

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